ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

ALK (L1196M) Kinase Assay

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Scientific Background:

ALK or anaplastic lymphoma kinase is a receptor tyrosine kinase that was originally identified as a member of the insulin receptor subfamily that acquires transforming capability when truncated and fused to NPM (nucleophosmin) in the t (2; 5) chromosomal rearrangement associated with ALCL Many chromosomal rearrangements leading to enhanced ALK activity have been described and are implicated in a number of cancer types (1). In the nervous system, ALK in the presence of ligand appears essential for axonal guidance, whereas in the absence of ligand, ALK expression can lead to developmental neuronal apoptosis (2).

1. Palmer R H, et al: Anaplastic lymphoma kinase: signalling in development and disease. Biochem J. 2009 May 27;420(3):345-61.

2. Allouche M: ALK is a novel dependence receptor: potential implications in development and cancer. Cell Cycle. 2007 Jul 1;6(13):1533-8

ADP-Glo™ Kinase Assay

Description

ADP-Glo™ Kinase Assay is a luminescent kinase assay that measures ADP formed from a kinase reaction; ADP is converted into ATP, which is converted into light by Ultra-Glo™ Luciferase (Fig. 1). The luminescent signal positively correlates with ADP amount (Fig. 2) and kinase activity (Fig. 3A). The assay is well suited for measuring the effects chemical compounds have on the activity of a broad range of purified kinases—making it ideal for both primary screening as well as kinase selectivity profiling (Fig. 3B). The ADP-Glo™ Kinase Assay can be used to monitor the activity of virtually any ADP-generating enzyme (e.g., kinase or ATPase) using up to 1mM ATP.

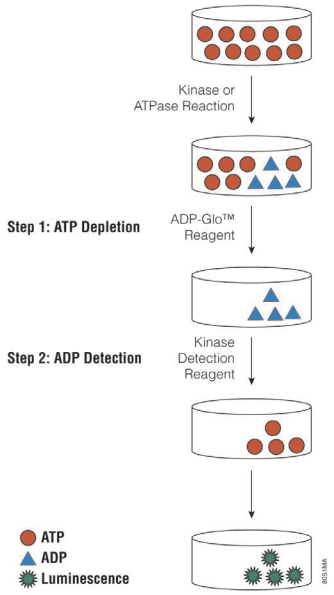

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

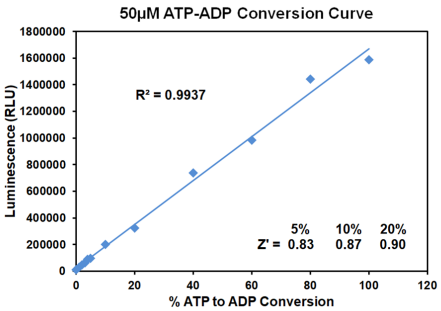

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction buffer.
- Add to the wells of 384 low volume plate:
  - ✓ 1 µl of inhibitor or (5% DMSO)
  - ✓ 2 µl of enzyme (defined from table 1)
  - ✓ 2 µl of substrate/ATP mix
- Incubate at room temperature for indicated time (See Figure 3).
- Add 5 µl of ADP-Glo™ Reagent.
- Incubate at room temperature for 40 minutes.
- Add 10 µl of Kinase Detection Reagent.
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1 second).

Table 1. Enzyme Titration. Data are shown as relative light units (RLU) that directly correlate to the amount of ADP produced. The correlation between the % of ATP converted to ADP and corresponding signal to background ratio is indicated for each kinase amount.

Enzyme, ng	240	120	60	30	15	7.50	3.75	1.88	0.94	0.47	0
Luminescence	1,176,070	1,006,286	927,539	699,078	494,964	282,969	157,571	68,339	35,351	17,475	6,444
S/B	183	156	144	108	77	44	24	11	5	3	1
% Conversion	58	50	46	34	24	13	7	3	1	0	0

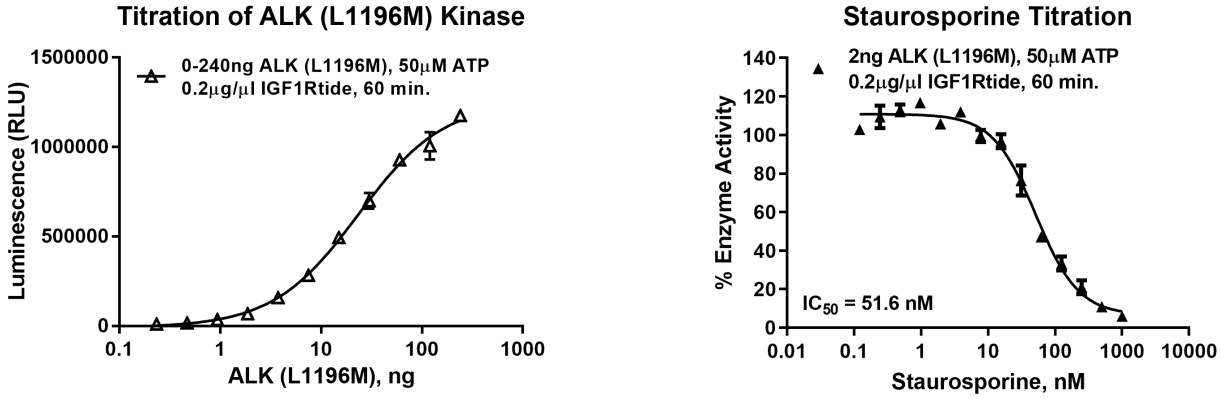

Figure 3. ALK (L1196M) Kinase Assay Development. (A) ALK (L1196M) enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2ng of ALK (L1196M) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
ALK (L1196M) Kinase Enzyme System	10µg	VA7372
	1mg	VA7373
ADP-Glo™ + ALK (L1196M) Kinase Enzyme System	1 Each	VA7374